

PROTOCOL FOR

Sterilex Ultra Disinfectant Cleaner Solution 1
Lot #: AM1-228A
8/18/15 Exp: 8/19/16

STUDY NUMBER

15-031-6

ACUTE INHALATION TOXICITY STUDY
OPPTS 870.1300 OECD 403 GUIDELINE

This study will be performed in compliance with EPA Good Laboratory Practices Guidelines and current OPPTS 870.1300 OECD 403 Guidelines. This guideline meets the requirements of the Federal Insecticide, Fungicide, Rodenticide Act (FIFRA) 40 CFR 160.

SPONSOR

Sterilex Corporation
111 Lake Front Drive
Hunt Valley, MD 21030

TESTING FACILITY

Tox Monitor Laboratories, Inc.
33 West Chicago Avenue
Oak Park, IL 60302

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STUDY TITLE: ACUTE INHALATION TOXICITY - OPPTS 870.1300 OECD 403 Guidelines
Sterilax Ultra Disinfectant Cleaner Solution 1
Lot #: AM1-228A 6/19/15 Exp: 6/19/16

1.0 PURPOSE OF STUDY:

The purpose of this study is to provide information on health hazards likely to arise from a short term exposure of Sterilax Ultra Disinfectant Cleaner Solution 1, Lot #: AM1-228A 6/19/15 Exp: 6/19/16 to young adult rats by the inhalation route according to OPPTS 870.1300, and OECD 403 Guidelines.

2.0 SPONSOR:

2.1 Name: Sterilax Corporation
2.2 Address: 111 Lake Front Drive
Hunt Valley, MD 21030
2.3 Sponsor's Representative: Edward Fu, Ph.D.

3.0 TESTING FACILITY:

3.1 Name: Tox Monitor Laboratories, Inc.
3.2 Address: 33 West Chicago Avenue
Oak Park, IL 60302
3.3 Study Director: Michael Kukulinski, B.S., L.A.T.G.

4.0 DATES:

4.1 Proposed Study Initiation Date: July 29, 2015
4.2 Proposed Study Completion Date: August 12, 2015
4.3 Proposed Final Report Date: August 14, 2015

5.0 TEST SUBSTANCE: Sterilax Ultra Disinfectant Cleaner Solution 1
Lot #: AM1-228A 6/19/15 Exp: 6/19/16

Sample Identification: Clear liquid

The test substance will be provided by the Sponsor. The test substance will be identified using the test substance identification number. The Sponsor is responsible for maintaining records of the test substance purity, strength and stability. This information will remain on file with the Sponsor. The Sponsor will also provide information on the chemical properties of the test substance, to insure safe handling of the material. The test substance will be inventoried and secured in a locked cabinet upon arrival. The Sponsor will be notified by email or telephone of the receipt of the test substance, and an email or letter will be sent confirming the amount and condition of the received material. The amount, date, identity of person(s) removing the dosing preparations, and the purpose for which each removal was made, shall be documented. At termination of study, a residual sample will be maintained at Tox Monitor Laboratories for any substance which required studies in excess of 28 days duration. Any remaining sample will be discarded.

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6.0 PERSONNEL:

Study Director: Michael Kukulinski, B.S., L.A.T.G.
Clinical Veterinarian: Thomas J. Welsh, D.V.M., Ph.D.
Quality Assurance: Robert F. Locke, M.S., L.A.T.G.
Consultant: Donald P. Waller, Ph.D., D.A.B.T.

7.0 TEST SYSTEM:

- 7.1 Species: Rat
7.2 Strain: Sprague-Dawley Derived
7.3 Number & Sex(s): 5 Males and 5 Females: The females will be nulliparous and nonpregnant.
7.4 Age/Weight Range: 8-12 weeks; 200-300 grams; the weight range will not exceed $\pm 20\%$ of the mean weight for each sex.
7.5 Source of Animals: Harlan Sprague Dawley, Indianapolis, Indiana
7.6 Justification for Selection of Test System: The Sprague-Dawley rat is the system of choice because, historically, it has been the preferred and most commonly used species for acute inhalation studies. All animals are experimentally naïve.
7.7 Procedure for Unique Identification of Test System: Upon arrival, each animal will be acclimated in a quarantine room until being assigned a permanent number for testing purposes. During the test animal selection process, each test animal will be assigned a test animal number, a stainless steel ear tag or color marking, unique to it within the population making up the study group. This number will also appear on a cage card visible on the front of each cage. The cage card will additionally contain the study number, test substance identification, treatment group number and dose level. Raw data records and specimens will also be identified by the unique test animal number.
7.8 Housing: Housed animals individually in suspended stainless steel cages. The housing is in compliance with the National Research Council's, Guide for the Care and Use of Laboratory Animals, National Academy Press 2011. The animal room will have a 12 hour light/dark cycle and will be kept clean and vermin free. Environmental temperature and humidity will be maintained at $22\text{ C} \pm 3^\circ\text{C}$ and 30-70 % respectively.
7.9 Quarantine/ Acclimation Procedures: Animals will be conditioned to the housing facility for at least a five day period prior to testing. During that time, the animals will be observed daily for signs of illness or death and all unusual observations will be reported to the Toxicologist/Study Director or Clinical Veterinarian. Animals will be examined during this period and approved for use by the Clinical Veterinarian or his representative prior to being placed on test. Any sickly animal will be eliminated prior to the test animal selection process at the onset of the study.
7.10 Food: Rodent Lab Diet 5001 will be available ad libitum.
7.11 Water: Tap water from water bottles will be provided ad libitum from arrival until termination.
7.12 Food & Water Quality: There are no known contaminants in the feed or water which are expected to influence the study. Water analysis is from the City of Chicago.

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8.0 EXPERIMENTAL DESIGN:

8.1 Treatment Levels and Number of Animals: Ten healthy animals (5 males/5 females) will be used for the test. The initial test material exposure concentration will be targeted at a level of slightly above 2 mg/L of air for a four hour period.

8.2 Sample Preparation and Generation: The test material will be used as submitted or as a workable dilution and will be generated into a Spraying System Co. (Wheaton, IL.) Model 1/4JSS atomizing nozzle assembly equipped with an appropriate size fluid cap. The nozzle will siphon the test substance from a reservoir and operate at an appropriate psi of air. The exposure will be conducted in a stainless steel chamber measuring 68.2 x 68.2 x 68.2 cm. Room air enters the top of the chamber and exits through the bottom. The aerosol enters this chamber through a hole in the rear of the chamber and passes immediately through an atomization plenum. The plenum will be used to improve the distribution of the aerosol within the chamber to reduce the size dispersity of the aerosol. The atomization plenum will be constructed of 6 inch diameter steel ducting and will be positioned at the top of the cubical portion of the chamber. The aerosol which does not escape the plenum will be received into the reservoir and recovered. Pilot study trails will be conducted and measured prior to test exposure to establish the stability of test article aerosol concentrations and MMAD particle size range (1-4 μ m). A minimum of two trials will be conducted and if test parameters are within 10% of each other, the generating system will be deemed acceptable. If pretest measurements are not within 10% of each other, additional measurements will be conducted to attain acceptable limits for the main test.

8.3 Exposure Chamber: Exposures will be conducted in a 400 L stainless steel exposure chamber designed by Spengler Engineering Associates of Cincinnati, Ohio. The exposure will be performed as either a "whole body exposure" or a "nose only exposure", depending upon the Sponsor's direction. Whole body exposures consist of the test animals being confined in an exposure cage inside the chamber, whereas in nose only exposures the test animals are placed into restrain tubes mounted on the exterior of the chamber with only their nose and mouth being exposed to the chamber atmosphere.

Preferred exposure route: Whole body exposure _____ Nose only exposure X

The chamber is equipped with an exhaust port which is connected to an exhaust fan, providing an adjustable air flow through the chamber. Air flow will be determined by the reading taken from a calibrated Dwyer Magnehelic pressure gauge, which measures the pressure drop across a defined inlet orifice in a stainless steel plate located in the air inlet duct of the chamber. The test material will be administered into the chamber at the junction of the air inlet port, which usually allows the test material and incoming air to mix evenly within the chamber at the top before being drawn down over the animals.

8.4 Concentration Measurements: Nominal concentrations will be calculated by dividing the total amount of test material used during the test period by the total air volume. Actual concentration determinations were taken every 30 minutes during the 4 hour period. The "actual" concentration of the study will be determined by gravimetric analysis based on samples taken at 30 minute intervals from the breathing zone of the animals. The analytical concentration will be determined by using a pre-weighed 25 mm Gelman glass fiber filter #66075 which will be placed into a filter holder manufactured by Gelman Instrument Co. The filter holder will be fitted with a stainless steel tube which was designed to be placed through a port in the chamber, which positions the filter into the breathing zone of the animals. The filter assembly will be attached via rubber tubing to an Anderson Sampler Pump, Model #10-709 and a measured volume of chamber atmosphere will be withdrawn, with the substances being collected on the 25 mm glass fiber filter.

The filter holder will be immediately withdrawn from the chamber and the filter will be removed and weighed. The increase in the filter weight, when divided by the volume of air sampled, yields the actual gravimetric concentration for the sample. This procedure will be repeated at 30 minute intervals for the duration of the exposure.

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8.5 Chamber Parameter Measurements:

Airflow: Airflow will be monitored continuously at thirty minute intervals during the exposure period.

Chamber Temperature: The inside chamber temperature will be monitored and recorded at thirty minute intervals during the exposure period. A temperature of 22 ± 2 °C will be maintained.

Chamber Humidity: Humidity inside the chamber will be monitored and recorded at thirty minute intervals during the exposure period. A relative humidity of 30-70% will be maintained if possible, but due to the nature of the test material, this may not be practical.

Particle Size Determination: Particle size distribution of the test material will be determined at 120 minutes during the exposure period using an Anderson 1 ACFM Ambient Sampler with preseparator. Particle size data consists of two major values, average size (MMAD) and geometric standard deviation (GSD). The MMAD gives the average size and the GSD indicates the range of particle size in the sample. The lower the GSD, the more uniform the sample. Particle size data is collected by drawing quantities of the test atmosphere into some form of collector. In this case, an Anderson Cascade Impactor will be used. A cascade impactor is designed with several stages stacked on top each other. Each stage has a collection plate and by varying the size and number of the holes in the plates, it is possible to collect only certain particle size range on a particular stage. The holes in the stages change the velocity of the air passing over the stage and the particles that are too large to stay airborne at a given velocity fall out and are collected on a filter which is determined prior to and after sample collection to determine the amount of particles collected on each stage. Most particle distributions follow a log-normal function. The particle size for each stage is determined by averaging the upper and lower size cutoff for the stage and log of that number is determined. The total amount of particles collected is determined and a cumulative percent of particles by stage is determined. This percentage can be plotted against the log size of the stages or converted to probits and then plotted on paper or with a regression program to obtain a best fit line. After data has been plotted, the log size where the line intercepts 50% (MMAD) on the graph is determined. The range of particles is determined by calculating the geometric standard deviation (GSD). The GSD is calculated by determining the log size where the line intercepts 84.1% on the graph. The anti-log of each value is taken and 84.1% value is divided by the 50% value and the result is the GSD.

8.6 Observations: The rats will be observed for mortality and pharmacotoxic signs at 1 hour, 2 hours and 4 hours after exposure and daily thereafter for 14 days. Body weights will be obtained at study initiation, 7 days and at 14 days post-administration. Gross necropsy examination will be performed on all survivors at the end of the 14-day observation period.

8.7 Pathology: Animals will be sacrificed by carbon dioxide asphyxiation at 14 days and will be subjected to a gross necropsy. Abnormalities will be recorded.

8.8 Toxicity Rating: The rating of the toxicity will be performed in accordance with the EPA/FIFRA Inhalation LC₅₀ Toxicity Rating.

9.0 RECORDS TO BE MAINTAINED:

All data generated during the study, except those that are generated as direct computer input, will be recorded directly, promptly and accurately in black ink on worksheets that will be bound during or at the conclusion of the study. All appropriate computer and machine output will be bound during, or at the conclusion of the study. Upon completion of the study and submission of the final report, all raw data, documentation, and other material necessary to reconstruct the study, will be stored permanently in the Tox Monitor Laboratories archives maintained by Quality Assurance, unless otherwise specified by the Sponsor. All changes or revisions, and reasons for such changes or revisions, to this protocol once it is approved, will be documented, signed by the Study Director and Sponsor, dated and maintained with the protocol. Quality Assurance will include protocol review, in life observations during the exposure interval, raw data review and final draft/report review.

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10.0 FINAL REPORT:

A draft final report may be sent to the Sponsor for comments prior to final approval. The final report will include, but is not limited to:

Summary - Brief description of the test performed and the results/conclusions. Any GLP deviations will be included as an Appendix to the Final Report.

Introduction - Description of test substance characteristics.

Methods - Description of the animal model (species/strain), route of administration and animal observations performed.

Results - Tabulation of response data for each individual animal for the observation time period including survival and any observed effects. Tables of data to include, food and body weight data, necropsy findings, and statistical treatment of results where appropriate. The Final Report will comply with PR Notice 86-5 and reporting requirements specified by OPPTS 870.1300 OECD 403 Guidelines.

11.0 ANIMAL WELFARE ACT PROVISIONS:

This experiment will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR). The Sponsor's signature on this protocol assures the Study Director that this procedure is not unnecessary duplication of a previous study. If at all possible, this protocol has been designed to avoid or minimize distress or pain to the test subjects. Any subjects that may experience severe or chronic distress or pain that is not able to be relieved will be euthanized as per direction of the Staff Veterinarian or Study Director in conformance with the above referenced regulation. The Sponsor will be notified in a timely manner should this situation occur.

12.0 QUALITY ASSURANCE - This study will be inspected and the final report reviewed by TML quality assurance personnel in accordance with QA Standard Operating Procedures and the pertinent government regulations.


13.0 REGULATORY REQUIREMENTS:

This study will be performed in compliance with OPPTS 870.1300 OECD 403 Guidelines. This guideline meets testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide (FIFRA) (7 U.S.C. 136, et seq.) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

Will this study be submitted to a Regulatory Agency? Yes ☒ No ☐

If so, which one? EPA

SIGNATURES:


SPONSOR'S REPRESENTATIVE
Edward Fu, Ph.D.

20 Jan 2015
DATE


STUDY DIRECTOR
Michael Kukulinski, B.S., L.A.T.G.

7/21/15
DATE